RELATIONSHIP OF INTRACELLULAR PROTEINS AND MUSCLE PIGMENTS TO THE TENDERNESS OF BOVINE MUSCLES [1, 2]

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THE relationship of intracellular protein composition and solubility characteristics to meat tenderness has been the object of considerable research. Wierbicki *et al.* (1954) postulated that the dissociation of actin and myosin could account for the postmortem tenderization of meat. Many workers have substantiated this theory. Partmann (1963) stated that the tenderness changes in the aging period were correlated with this dissociation process. Weinberg and Rose (1960) found increased extractability of myosin and actomyosin in post-rigor chicken breast muscle. Khan and van den Berg (1964) also reported increased myofibrillar protein solubility during postmortem aging. Hegarty, Bratzler and Pearson (1963) reported that increased tenderness was associated with increased solubility.

However, conflicting results have also been reported. Wierbicki *et al.* (1956) stated that the postmortem dissociation of actomyosin probably played an insignificant role in meat tenderization. Goll, Henderson and Kline (1964) also reported no significant difference in the amount of sarcoplasmic or myofibrillar protein extractability with postmortem aging.

This study was initiated to further clarify the relationship of intracellular protein solubility characteristics to muscle tenderness.

Materials and Methods

Experimental Animals. The *longissimus dorsi* muscles from 12 yearling steers, classified as tender or less tender on the basis of Warner-Bratzler shear values, were utilized in this study. The selection and treatment of experimental samples has been reported previously (McClain *et al.*, 1965).

Extraction Procedure. The extraction procedure utilized was adopted from the methods of Fisher (1963) and Hegarty *et al.* (1963). All fractionation procedures were carried out at 2° C. A 5-gm. sample was placed into a Virtis homogenizing flask with eight volumes of distilled water and mixture homogenized at a setting of 20 for 3 minutes. After blending, the mixture was centrifuged for 30 min. at 3,500 x g. The supernatant was then filtered through a coarse sintered glass filter. The blender jar was rinsed with an additional eight volumes of distilled water and the rinse solution poured into the centrifuge tube with the residue. This mixture was again centrifuged for 30 min., the supernatant filtered and the extracts combined. This fraction was designated as solution A (water soluble protein). To a 40 ml. aliquot of solution A was added 20 ml. of 10% trichloracetic acid (TCA). The TCA filtrate was designated fraction B (A—B =C; sarcoplasmic protein).

The residue from the water extract was returned to the homogenizing flask and extracted with 1.0 M KCl as above (solution D=soluble myofibrillar protein). The residue remaining from the 1.0 M KCl extraction was then extracted with 0.1 M NaOH (solution E=insoluble fibrillar protein. The residue remaining from this extraction was designated as stroma protein (fraction F). The soluble myofibrillar proteins were further fractionated by dilution (Weinberg and Rose, 1960) into three fractions: (1) insoluble at 0.5 M KCl, (2) insoluble at 0.2 M KCl and (3) insoluble at 0.05 M KCl.

Nitrogen and Pigment Determinations. All nitrogen analyses were performed by microkjeldahl digestion and Nesslerization as outlined by Hawk, Oser and Summerson (1954). The total pigment content of the water soluble extracts was determined according to the method of Wierbicki *et al.* (1955). Samples from the *l. dorsi* muscles were ashed and the iron content determined by the method of Barkan and Walker (1940).

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TABLE 1. MEAN NITROGEN[a] COMPOSITION OF *LONGISSIMUS DORSI* MUSCLES

	Non-protein	Sarco-plasmic	Soluble myofibrillar	Insoluble myofibrillar	Stroma	Total	Total nitrogen	Percent recovery
	B	C	D	E	F	B,C,D,E,F		
Mean[b]	3.71	5.32	5.66	15.96	1.78	32.43	33.06	98
S.D.	0.51	1.19	0.84	2.83	0.50	5.63	3.19	..

[a] mg. N/gm. wet weight muscle.
[b] Average of 12 animals.

Results and Discussion

Protein Extraction. Table 1 shows the mean nitrogen composition of the *l. dorsi* muscles. The values for the nonprotein (B), total fibrillar (D+E) and total nitrogen fractions are in good agreement with those reported by Hegarty *et al.* (1963). The values for the sarcoplasmic and soluble myofibrillar fractions, however, are lower than those reported by the above workers (table 1). The sodium hydroxide insoluble fraction (F) appears to be too high to represent stroma protein alone, and was probably contaminated by denatured fibrillar proteins. The average recovery of nitrogen utilizing the above extraction procedure was 98%.

The sarcoplasmic and soluble myofibrillar protein content was similar in the two groups (table 2), indicating that the tenderness differences were not related to protein solubility. The tender muscles tended to have a lower content of the 0.05 M KCl insoluble or myosin fraction (Fisher, 1963) than the less tender group. These differences were approaching significance at the 5% level. A significant difference ($P<.05$) between the tender and less tender muscles was found in the 0.5 M KCl insoluble fraction. When the nitrogen from the 0.2 and 0.5 M fractions was combined, a highly significant difference ($P<.01$) was found between the two tenderness groups. Weinberg and Rose (1960) reported that the precipitation of actomyosin was complete at an ionic strength of 0.20 and 0.25. However, Fisher (1963) indicated that the fraction precipitated in 0.5 M KCl probably resulted from protein configurational changes or interactions caused by the change in ionic strength. Therefore, it is probable that incomplete or co-precipitation occurred between the 0.5 and 0.2 M KCl insoluble fractions, and that the values obtained for the combined fractions more nearly represents the actomyosin content of the samples.

The increased 0.2 plus 0.5 M KCl insoluble fractions in the extracts from the tender muscles may have resulted from the reformation of the actomyosin complex in the extracting medium. Therefore, no unequivocal conclusions can be reached regarding the degree of dissociation of actomyosin. However, the increase in these combined fractions and the decrease in the 0.05 M KCl fractions suggests that there was an increase in the amount of actin extracted from the *l. dorsi* muscles of the tender group (Weinberg and Rose, 1960). These results would tend to substantiate the conclusion that variations in tenderness are related to the dissociation of the actomyosin complex.

Muscle Pigments. In the course of this study, color differences in the water extracts were noted between the tender and less tender

TABLE 2. MEAN VALUES FOR SARCOPLASMIC AND MYOFIBRILLAR NITROGEN[a] FROM *LONGISSIMUS DORSI* MUSCLES OF TWO TENDERNESS GROUPS[b]

	Shear** values[c]	Sarco-plasmic	Soluble myofibrillar	Insoluble* 0.5 M KCl	Insoluble 0.2 M KCl	Insoluble** 0.5 M+ 0.2 M KCl	Insoluble 0.05 M KCl	Stroma
Tender								
Mean	8.37	5.23	5.94	1.35	1.14	2.49	0.79	1.87
S.D.	0.49	0.15	0.50	0.18	0.28	0.11	0.40	0.39
Less tender								
Mean	15.00	5.41	5.36	0.73	1.08	1.83	1.21	1.70
S.D.	0.97	1.64	0.90	0.17	0.30	0.04	0.12	0.42

[a] mg. N/gm. wet weight muscle.
[b] Average of six animals in each group.
[c] Kilograms of shear force on a 2.5 cm. core.
* Means significantly different at the 0.05 level.
** Means significantly different at the 0.01 level.

TABLE 3. MEAN VALUES FOR TOTAL PIGMENT AND IRON CONTENT FROM *LONGISSIMUS DORSI* MUSCLES OF THE TWO TENDERNESS GROUPS

	Total pigment**	Iron*
	mg./gm.	mg. Fe/100 gm.
Tender		
Mean	1.81	3.93
S.D.	0.03	0.56
Less tender		
Mean	2.37	5.82
S.D.	0.02	2.10

* Means significantly different at 0.05 level.
** Means significantly different at 0.01 level.

muscles. The mean values for the total pigment concentration of the two tenderness groups are shown in table 3. A significantly ($P<.01$) greater amount of total pigment was found in the water extracts from the less tender *l. dorsi* muscles. Hershberger *et al.* (1951) and Wierbicki *et al.* (1955) also reported an inverse relationship between myoglobin content and tenderness.

To further substantiate the findings of the present study, the iron content of the muscles was determined. A significantly ($P<.05$) greater amount of iron was evident in the less tender muscles (table 3). Thus, these results strongly suggest a relationship between concentration of muscle pigment and tenderness.

Summary

The *l. dorsi* muscles from 12 yearling steers, classified as tender and less tender on the basis of Warner-Bratzler shear values, were utilized in this study. The sarcoplasmic and myofibrillar proteins were extracted. Fibrillar proteins were further fractionated by ionic strength dilution. Total pigments and iron content were also determined. Less tender muscles tended to have lower quantities of soluble myofibrillar protein, however, differences were not statistically significant. No differences were found in sarcoplasmic protein. The combined fractions insoluble at 0.5 and 0.2 M KCl were significantly greater in the tender muscles. The water extracts from the tender muscles had a significantly lower total pigment and iron content than that from the less tender group.

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